p34^{cdc2} Acts As a Lamin Kinase in Fission Yeast

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Abstract. The nuclear lamina is an intermediate filament network that underlies the nuclear membrane in higher eukaryotic cells. During mitosis in higher eukaryotes, nuclear lamins are phosphorylated by a mitosis-specific kinase and this induces disassembly of the lamina structure. Recently, p34^{cdc2} protein kinase purified from starfish has been shown to induce phosphorylation of lamin proteins and disassembly of the nuclear lamina when incubated with isolated chick nuclei suggesting that p34^{cdc2} is likely to be the mitotic lamin kinase (Peter, M., J. Nakagawa, M. Dorée, J. C. Labbe, and E. A. Nigg. 1990*b*. *Cell.* 45:145–153). To confirm and extend these studies using genetic techniques, we have investigated the role of p34^{cdc2} in lamin phosphorylation in the fission yeast. As fission yeast lamins have not been identified, we have introduced a cDNA encoding the chicken lamin B_2 protein into fission yeast. We report here that the chicken lamin B_2 protein expressed in fission yeast is assembled into a structure that associates with the nucleus during interphase and becomes dispersed throughout the cytoplasm when cells enter mitosis. Mitotic reorganization correlates with phosphorylation of the chicken lamin B_2 protein by a mitosis-specific yeast lamin kinase with similarities to the mitotic lamin kinase of higher eukaryotes. We show that a lamin kinase activity can be detected in cell-free yeast extracts and in p34^{cdc2} immunoprecipitates prepared from yeast cells arrested in mitosis. The fission yeast lamin kinase activity is temperature sensitive in extracts and immunoprecipitates prepared from strains bearing temperature-sensitive mutations in the *cdc2* gene. These results in conjunction with the previously reported biochemical studies strongly suggest that disassembly of the nuclear lamina at mitosis in higher eukaryotic cells is a consequence of direct phosphorylation of nuclear lamins by p34^{cdc2}.

THE fission yeast, *Schizosaccharomyces pombe*, has been a useful experimental system for the study of the eukaryotic cell cycle as numerous mutants deficient in cell cycle control have been identified (for review see reference 22). A central controlling element is the protein kinase p34^{cdc2}, activation of which is required for initiation of mitosis. Recent work has shown that *cdc2* has been highly conserved throughout evolution and is required for initiation of mitosis in all eukaryotic cells (for review see reference 36). Entry into mitosis is characterized by profound changes in cellular organization (for review see reference 25), and it is important to determine how *cdc2* activation causes these mitotic events.

It is likely that protein phosphorylation plays an important role in promoting mitotic changes in structure as entry into mitosis is accompanied by a general increase in protein phosphorylation (16, 21, 23, 28). One prominent mitotic substrate is the nuclear lamina, a structure composed of polymers of intermediate filament type proteins called lamins. During interphase the nuclear lamins are found in an insoluble matrix that underlies the inner nuclear membrane. During mitosis the nuclear lamina depolymerizes and lamins are either found free in the cytoplasm (A-type lamins) or associated with membranes (B-type lamins). These changes correlate with lamin phosphorylation by a mitosis-specific kinase (6, 26, 38, 44; and for reviews see references 7, 35).

We wished to investigate whether fission yeast contain a mitosis-specific lamin kinase, and if so, what role p34^{cdc2} plays in its activity. Attempts to identity fission yeast nuclear lamin proteins have not been successful although proteins immunologically related to lamins have been identified in the budding yeast *Saccharomyces cerevisiae* (5). To investigate whether fission yeast have a lamin kinase, we introduced a cDNA encoding a lamin protein from higher eukaryotes into fission yeast. We used a cDNA encoding the chicken lamin B_2 protein (20, 48) the phosphorylation of which has been studied extensively (31, 40). Here we report that chicken lamin B_2 protein expressed in fission yeast forms a ringlike structure associated with the nucleus. The chicken lamin B_2 protein is phosphorylated, and phosphorylation increases when cells are blocked in mitosis. Phosphorylation of the chicken protein correlates with replacement of the nuclear ring structure with a pattern of dispersed punctate staining. The mitotic sites of chicken lamin B_2 phosphorylation differ from those phosphorylated during interphase, and are a subset of those phosphorylated by a lamin kinase in

metaphase chick cells (40). We conclude that fission yeast contain a mitosis-specific lamin kinase similar to the lamin kinase of higher eukaryotes.

The yeast lamin kinase activity can be detected in vitro in crude extracts and $p34^{cdc2}$ immunoprecipitates prepared from yeast arrested in mitosis. The lamin kinase activity is temperature sensitive in extracts and $p34^{cdc2}$ immunoprecipitates prepared from a temperature-sensitive cdc2 mutant. Thus, we proposed that the $p34^{cdc2}$ kinase directly phosphorylates the chicken lamin B_2 expressed in yeast. As $p34^{cdc2}$ controls entry into mitosis in all eukaryotic cells, it seems likely that such a mechanism determines lamina disassembly in higher eukaryotes as well, as has been proposed (40). Recent work has shown that $p34^{cdc2}$ purified from starfish can directly phosphorylate lamins and induce disassembly of the nuclear lamina in isolated nuclei (40). Our results confirm and extend these findings, showing that mutation of *cdc2* has a direct affect on lamin kinase activity.

We discuss the possibility that the nuclear structure we observe in fission yeast expressing the chicken lamin B_2 protein could form as a result of interactions between the chicken lamin B_2 protein and components of an endogenous yeast karyoskeleton. The mitotic reorganization of this structure indicates that the yeast nucleus may undergo a process analogous to mitotic disassembly of the nuclear lamina. This was unexpected, as the yeast nuclear membrane does not breakdown during mitosis. If yeast have a karyoskeleton, this type of reorganization may be required to accommodate the rapid changes in nuclear structure necessary for nuclear division. This would be consistent with a growing body of evidence that nuclear structures are fundamentally similar in higher and lower eukaryotes.

Materials and Methods

Plasmids and Yeast Strains

Media and standard yeast techniques were as described by Mitchison (27). The strain referred to as wild-type is 972h- (17). The plasmid pEVP-LamB2 (Fig. 1 *A*) was constructed using an Eco RI fragment containing a 2-kb cDNA encoding the chicken lamin B_2 protein (48). The ends of this fragment were filled in and subcloned into a filled-in Bam HI site in the plasmid pEVP11 (42). This plasmid was introduced by transformation into *leu1-32* strain (17) and leucine prototrophs were selected. To isolate a strain in which the plasmid had integrated stably, leu^+ transformants were grown to saturation in yeast extract media, conditions that are nonselective for the leucine marker on the plasmid. This culture was plated on minimal plates lacking leucine and leu^+ colonies were selected, streaked on yeast extract plates and then replica plated to minimal plates lacking leucine. In most cases, only a fraction of the colonies on the yeast extract plates were able to grow on plates lacking leucine, indicating that the leucine marker was episomal. However, in one strain all the colonies on the yeast extract plate gave rise to viable colonies on minimal plates indicating that the leucine marker was stably inherited. DNA was prepared from this strain, and Southern blotted using the chicken lamin B_2 cDNA and a fragment of the *adh* promoter as probes. These experiments confirmed that the plasmid pEVP-LamB2 had integrated at the *adh* locus to give the structure shown in Fig. 1 *B*. This strain was designated LamB2.

To obtain chicken lamin B_2 expressing strains that could be arrested in mitosis, the strains $nda3^{cs}$LamB2 and $cdc13^{ts}$LamB2 were constructed. LamB2 was crossed to *cdc*13-117 *leu1*-32 (32, 37) or *nda3*-KM311 *leu1*-32 (47). As the leucine marker in LamB2 is closely linked to the chicken lamin B_2 coding sequences (Fig. 1 *B*), all leu^+ progeny of this cross must contain the chicken lamin B_2 gene. Thus, the progeny of the above crosses were screened for colonies that were leu^+ and either temperature sensitive (in the case of $cdc13^{ts}$) or cold sensitive (in the case of $nda3^{cs}$). The presence of the chicken lamin B_2 protein was confirmed by immunofluorescence and Western blotting (data not shown).

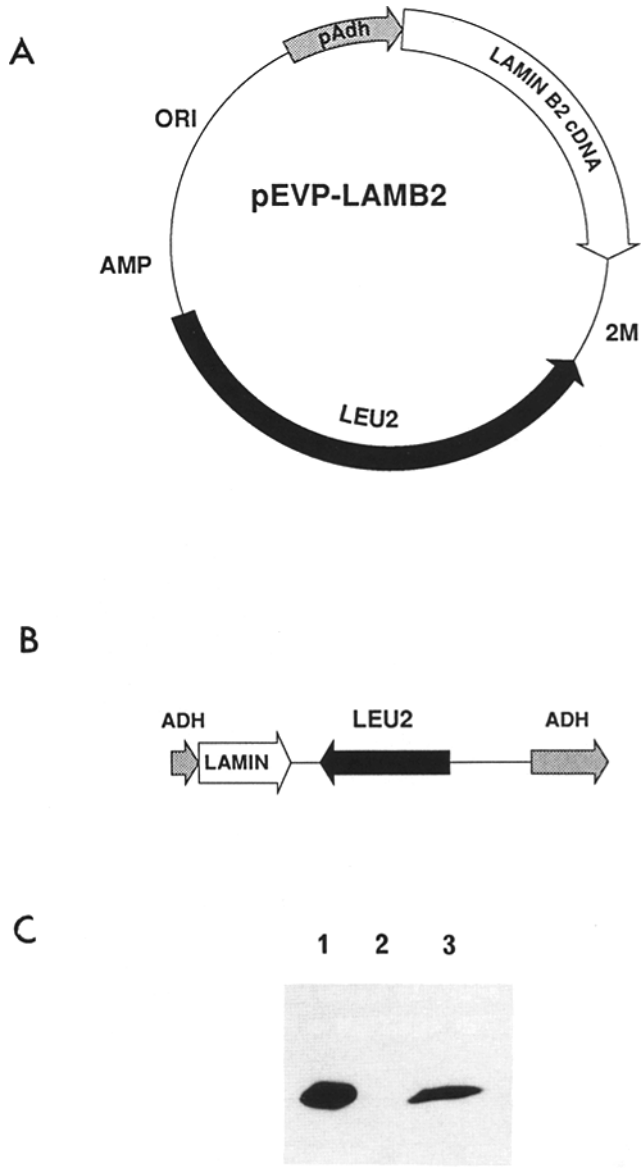


Figure 1. Expression of chicken nuclear lamin B2 in fission yeast. (*A*) Plasmid pEVP-LamB2 constructed to express chick nuclear lamin B2 in fission yeast. See Materials and Methods for complete description. (*B*) Organization of pEVP-LamB2 sequences after stable integration of the plasmid at the fission yeast *adh* locus. (*C*) Western blot using anti-chicken lamin B_2 antibodies. Lane *1*, whole cell extract from chick DU249 cells; lane *2*, extract from yeast cells transformed with pEVP11; lane *3*, extract from yeast cells transformed with pEVP-LamB2.

Preparation of Protein Extracts, Immunoprecipitation, and Western Blotting

Approximately 2×10^7 logarithmically growing yeast cells (O.D. $_{595}$ of 0.2–0.4) were pelleted by low-speed centrifugation, washed once with cold STOP buffer (0.9% NaCl, 5 mM NaF, 10 mM EDTA, 1 mM NaAzide), transferred to a 3-ml Falcon tube and then resuspended in 20 μl of HE buffer (50 mM Hepes, pH 7.9, 5 mM EDTA, 5 mM EGTA, 50 mM NaF, 50 mM β-glycerophosphate, 1 mM DTT, 1 μg/ml leupeptin and pepstatin, 1 mM PMSF). 1 g of glass beads was added to the suspension, and the mixture was vortexed vigorously for 1 min. For Western blotting experiments, 1 ml of SDS-PAGE sample buffer was added and the sample was incubated at 80°C for 5 min. The buffer was collected and spun in an Eppendorf centrifuge (Brinkman Instruments Inc., Westbury, NY) for 10 min to pellet cel-

lular debris. For immunoprecipitations, 200 μl of HE + 1% SDS was added and the cells were incubated at 80°C for 5 min. The beads were washed with 1 ml RIPA buffer (150 mM NaCl, 50 mM Tris, pH 8.0, 1% Triton X-100) and then spun for 10 min in an Eppendorf microfuge to pellet debris. To analyze the extractability of the chicken lamin B_2 protein, cells were lysed with glass beads as described above in 20 μl of HE buffer, and then incubated in either 1 ml of LS buffer (20 mM Tris, pH 8.0, 5 mM $MgCl_2$, 2.5 mM KCl, 1 mM PMSF) or 1 ml of HS buffer (LS + 1 M NaCl, 1% Triton X-100) for 20 min at room temperature on a rotary shaker. At the end of the incubation, the liquid was removed and spun for 15 min in an Eppendorf microfuge. SDS, DTT, and glycerol were added to the supernatant to a final concentration of 2%, 1 mM and 10%, respectively. These samples were designated "supernatant fractions." A volume of either LS or HS supplemented with SDS, DTT, and glycerol equal to the volume of the relevant supernatant was added to each pellet. These samples were designated "pellet fractions." All the samples were then incubated at 80°C for 5 min. The fractions containing HS were desalted by passing them through a Sephadex G-50 spin column equilibrated with LS + SDS, DTT, and glycerol. Just before electrophoresis bromophenol blue was added to each sample to a final concentration of 0.1%.

Western blots were done using Immobilon (Amersham International, Amersham, UK) according to the manufacturer's instructions. Blots were probed with cell culture supernatant from the monoclonal mouse cell line L4, which produces monoclonal antibody against chicken lamin B_2 (20) and developed using alkaline phosphate-conjugated secondary antibody directed against mouse IgG (Sigma Chemical Co., Poole, England). Chicken lamin B_2 immunoprecipitations were done using 1 μl of ascites fluid from the E-3 mouse anti-chicken lamin B_2 monoclonal line (20) for every 1 ml of yeast extract prepared as described above. To precipitate the antigen-antibody complex the extract was incubated first with rabbit anti-mouse IgG (Sigma Chemical Co.) for 30 min and then adsorbed to protein A-Sepharose beads for 45 min.

In vivo labeling with ^{32}P-inorganic phosphate was done as described in Gould and Nurse (8). Phosphoamino acid analysis was done as described in Cooper et al. (3). Tryptic peptide-mapping experiments were performed as described in Peter et al. (40). ^{32}P-labeled lamins from interphase and metaphase DU249 chicken cells were isolated as described in Peter et al. (40). Tissue culture and whole cell extract preparation of DU249 cells were carried out as described in Nakagawa et al. (31).

Lamin Kinase Assays in Crude Mitotic Extracts

Native yeast extracts for lamin kinase assays were prepared from strains containing *nda3*-KM311 mutation (14) which had been arrested in mitosis by incubation for 5 h at 20°C. High levels of $p34^{cdc2}$ histone kinase activity can be detected in native extracts prepared from cells arrested in this manner and 50–60% of the cells have condensed chromosomes (29). To obtain a mitotic extract with a temperature-sensitive $p34^{cdc2}$ histone HI kinase (29), the strain *nda3*-KM311 *cdc2*-L7 was generated by crossing *cdc2*-L7 (37) and *nda3*-KM311 and selecting recombinants that were both cold and temperature sensitive. The double mutant was viable at temperatures between 29° and 33°C. Extracts for kinase assays were prepared as described in Moreno et al. (29). Briefly, 2×10^9 cells were washed in STOP buffer (see above), transferred to a Falcon tube (Falcon Labware, Oxnard, CA), resuspended in 20 μl of HB buffer (60 mM β-glycerophosphate, 15 mM *p*-nitrophenylphosphate, 25 mM MOPS, pH 7.2, 15 mM $MgCl_2$, 15 mM EGTA, 1 mM DTT, 0.1 mM sodium orthovanadate, 1 mM PMSF, 20 μg/ml leupeptin, pepstatin, and aprotinin), and lysed by vortexing with 2.5-g acid-washed glass beads. The beads were washed with 1 ml HB buffer and the extract was centrifuged for 15 min in an Eppendorf microfuge (Brinkman Instruments, Inc.) to pellet debris.

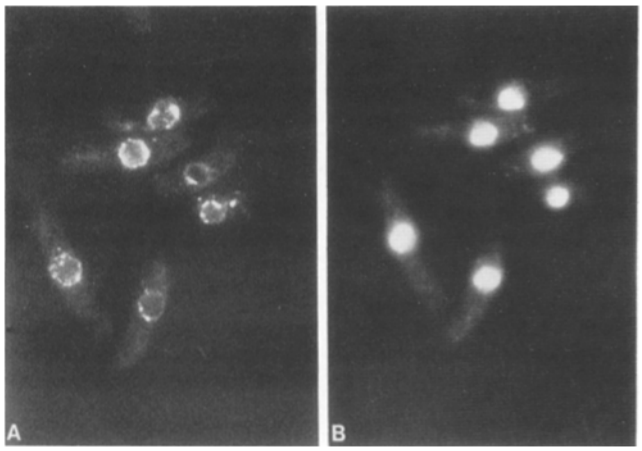


Figure 2. Chicken lamin B_2 expressed in fission yeast forms a ringlike structure associated with the nucleus. (*A*) Indirect anti-chicken lamin B_2 immunofluorescence of asynchronous *LamB2* cells; (*B*) The same field shown in *A* stained with the DNA-specific dye, DAPI.

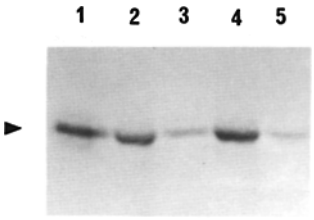


Figure 3. Subcellular distribution of chicken lamin B_2 expressed in fission yeast. *LamB2* cells were lysed as described in Materials and Methods and then extracted for 20 min with the indicated buffers. After the extraction, where indicated the samples were fractionated by centrifugation to obtain pellets and supernatants. Lane *1*, samples extracted with SDS-PAGE sample buffer; lane *2*, pellet from sample extracted with buffer containing 1 M NaCl, 1% Triton X-100; lane *3*, supernatant from the same extraction shown in *B*; lane *4*, pellet from sample extracted with a low ionic strength buffer; lane *5*, supernatant from the same extraction shown in lane *4*. For composition of buffers see Materials and Methods.

Lamin substrate for in vitro phosphorylation was prepared using a bacterial strain containing the chicken lamin B_2 cDNA subcloned in the expression plasmid pAR3038 (41). This plasmid and preparation of chicken lamin B_2 protein will be described in more detail elsewhere (Heitlinger, E., M. Peter, M. Haener, A. Lustig, U. Aebi, and E. A. Nigg, manuscript submitted for publication). Briefly, chicken lamin B_2 expression was induced by infection with bacteriophage λ CE6 as described in Rosenberg et al. (41). 2 h after induction the cells were harvested by centrifugation, resuspended in RIPA buffer, and lysed by sonication. The lysate was centrifuged for 10 min and chicken lamin B_2 was immunoprecipitated from the supernatant as described above. The protein A-Sepharose beads from the immunoprecipitation were washed twice with RIPA + 20 mM NaF + 50 mM β-glycerophosphate, twice in HB buffer and then resuspended in 40 μl HB buffer and 5 μl yeast extract. As a control, immunoprecipitations using extracts from uninduced bacteria were treated in the same manner (data not shown). The beads and extract were preincubated for 10 min at either 25° or 35.5°C. The kinase reaction was started by the addition of unlabeled ATP to a final concentration of 10 μM and 15–30 μCi of $\gamma^{32}P$-ATP (Amersham International). The reaction was incubated for 15 min and then stopped with 1 ml of RIPA buffer. The protein A-Sepharose beads were washed twice with RIPA buffer, once with PBS and then boiled in SDS-PAGE sample buffer before gel electrophoresis. In each experiment, Histone HI kinase activity was measured in parallel as described in Moreno et al. (1989). Where indicated, bacterially synthesized $p13^{suc1}$ prepared as described (2, 29) was added to a final concentration of 0.5 μM.

Lamin Kinase Assays in $p34^{cdc2}$ Immunoprecipitates

$p34^{cdc2}$ was immunoprecipitated from crude yeast extracts prepared as described above using the previously described antiserum AB4711 (8) as described in Gould et al. (9). 1-ml extracts from 3×10^8 cells were incubated with 5 μl of serum for 30 min, followed by incubation with protein A-Sepharose for 1 h. The protein A-Sepharose was then washed extensively with HB buffer (see above). For kinase assays as much residual HB buffer as possible was removed and the immunoprecipitates were preincubated at the desired temperature for 15 min. The reaction was started by the addition of 10 μl of lamin buffer containing 0.2 μg/ml solubilized bacterially produced chicken lamin B_2, 150 mM NaCl, 1 mM DTT, 1 mM EGTA, 15 mM $MgCl_2$, 50 μm ATP and 10 microcuries $\gamma^{32}P$-ATP (Amersham International) and incubated for 15 min. Soluble chicken lamin B_2 was prepared from bacterial extracts using a method described in detail elsewhere (Heit-

linger et al., op. cit., manuscript submitted for publication). The reaction was stopped by the addition of 20 μl of SDS-PAGE sample buffer. ^{32}P-labeled chicken lamin B_2 was visualized by PAGE and subsequent autoradiography.

Immunofluorescence and Diaminophenylimine Staining

Cells were fixed in fresh 3.5% paraformaldehyde for 30 min and then processed as described in Hagan and Hyams, 1988 (10). The L4 monoclonal antibody (undiluted tissue culture supernatant) described above was the primary antibody and FITC-conjugated goat anti-mouse (Sigma Chemical Co.) was the secondary antibody. After incubation with antibody, cells were dried onto coverslips and then mounted in a drop of 50% glycerol containing 1 μg/ml diaminophenylimine (DAPI)[1] and 1 μg/ml paraphenylene diamine as an antibleaching agent.

Results

Expression of Chicken Lamin B_2 in Fission Yeast

To express the chicken lamin B_2 protein in fission yeast the plasmid pEVP-LamB2 (shown schematically in Fig. 1 *A*) was constructed (see Materials and Methods). The plasmid contains a cDNA encoding the chicken lamin B_2 protein (48) under the control of the *S. pombe adh* promoter, sequences required for replication in bacteria and yeast and the *S. cerevisiae* LEU2 gene as a selectable marker (42). This plasmid was introduced into a *leu1-32* strain, and colonies capable of growing on minimal media were selected. Protein extracts from this strain were probed by immunoblotting with a monoclonal antibody against the chicken lamin B_2 protein (20) and compared to protein isolated from cultured chick DU249 cells. As shown in Fig. 1 *C*, a protein the size of the chicken lamin B_2 protein (lane *1*) can be detected in yeast transformed with the plasmid pEVP-LamB2 (lane *3*). No such protein is detected in a strain transformed with a control plasmid (lane *2*). Previously it has been observed that lamin B_2 protein isolated from chicken cells migrates more rapidly than the primary translation product of the cDNA (48, 49). This is likely to be the result of isoprenylation, a posttranslational modification that appears to be required to target lamins to the nuclear envelope (Kitten and Nigg, unpublished data; 15, 18, 49). As the mobility of the chicken lamin B_2 protein isolated from yeast is identical to that of the modified chicken protein, it seems likely that fission yeast can also modify lamins in this fashion.

To obtain a strain that expressed the chicken lamin B_2 protein uniformly, the original transformants were screened for variants in which the plasmid had integrated stably into the yeast chromosomes (see Materials and Methods). Southern blot analysis of a stable variant showed that the plasmid had integrated at the *adh* locus to give the structure shown schematically in Fig. 1 *B* (data not shown). As indicated, the chromosome in this strain has two tandem copies of the *adh* promoter, one of which remains linked to the *S. pombe adh* gene and a second copy that drives expression of the chicken lamin B_2 cDNA. We will refer to this strain as *LamB2*.

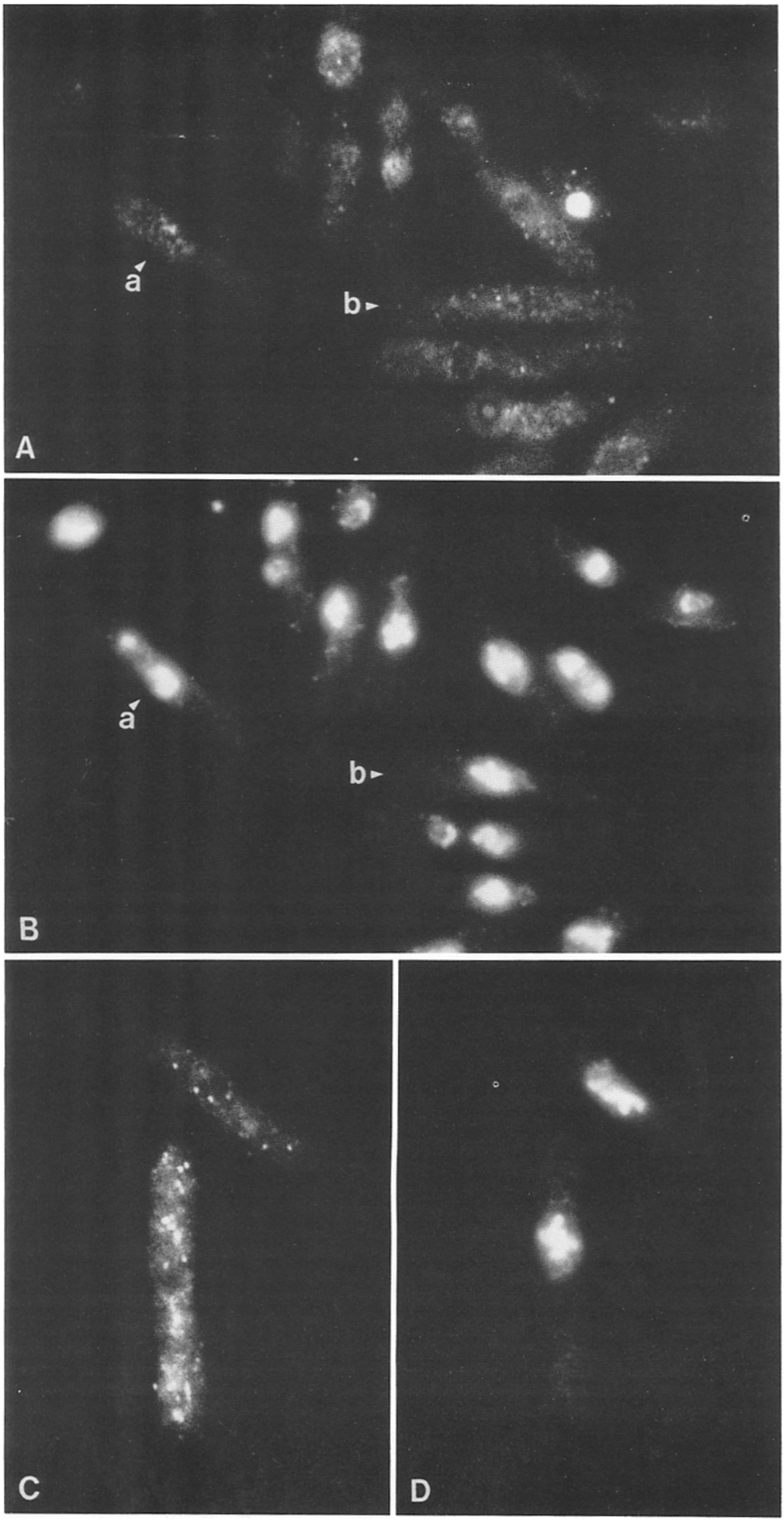
Cellular Localization of Chicken Lamin B_2 Expressed in Fission Yeast

To investigate the cellular localization of the chicken protein in fission yeast, *LamB2* cells were fixed and processed for immunofluorescence (10), and probed with a monoclonal antibody against chick lamin B_2 (20). The results of these experiments are shown in Fig. 2. Fig. 2 *A* shows the location of the chicken lamin B_2 proteins, and Fig. 2 *B* shows the same cells stained with the DNA-specific dye DAPI. As can be seen, the chicken lamin B_2 proteins are found in a ring-like structure associated with the nucleus. No staining was detected in cells that had not been transformed with pEVP-LamB$_2$ (data not shown). To investigate the cellular location of the chicken lamin B_2 protein biochemically, cellular fractionation experiments were carried out. *LamB2* cells were lysed and then extracted either with a low ionic strength buffer (Fig. 3, lanes *4* and *5*), or a buffer containing 1% Triton X-100 and 1 M NaCl (Fig. 3, lanes *2* and *3*). In addition, one sample was boiled in SDS-PAGE sample buffer to analyze total protein (Fig. 3, lane *1*). After a 20–30-min incubation, the extract was separated by centrifugation into soluble and insoluble fractions, each of which was boiled in SDS-PAGE sample buffer, and then fractionated by gel-electrophoresis. The gel was then electro-blotted and probed with a monoclonal antibody to the chicken lamin B_2 protein. The bulk of the chicken lamin B_2 protein remained in the insoluble fraction (Fig. 3, lane *2*, high salt/detergent pellet; lane *4*, low salt pellet) regardless of the extraction procedure. Only a small fraction of the chicken lamin B_2 protein was solubilized as a result of either extraction procedure (Fig. 3, lane *3*, high salt/detergent supernatant; lane *5*, low salt supernatant). Thus, the chicken lamin B_2 protein expressed in fission yeast appears to form a structure with solubility properties similar to those of the nuclear lamina of higher eukaryotes (6).

In higher eukaryotes the nuclear lamina disassembles as cells enter mitosis. To investigate whether the structure we observe in fission yeast undergoes a mitotic rearrangement we performed immunofluorescence studies on *LamB2* cells arrested in mitosis. To arrest cells in mitosis we used the cold-sensitive mutant *nda3*-KM311 which blocks in mitosis because of a defect in the beta-tubulin protein (14). This strain was crossed with *LamB2* (see Materials and Methods) to generate the strains *nda3^{cs} LamB2*. This strain was arrested in mitosis and the cellular location of the chicken lamin B_2 protein was examined by indirect immunofluorescence as described above.

1. *Abbreviation used in this paper*: DAPI, diaminophenylimin.

Figure 4. Redistribution of chicken lamin B_2 during mitosis in fission yeast. Anti-chicken lamin B_2 immunofluorescence and DAPI staining of *nda3^{cs}LamB2* cells arrested in mitosis by incubation at 20°C for 5 h. (*A* and *C*) Anti-chicken lamin B_2 immunofluorescence; (*B*) cells shown in *A* stained with DAPI; (*D*) cells shown in *C* stained with DAPI. (*A* and *B*) Show an average field of cells. Arrow *a* indicates a cell in which chicken lamin B_2 remains associated with the nucleus; arrow *b* indicates a cell in which the chicken lamin B_2 becomes widely distributed throughout the cytoplasm. *C* and *D* show a good example of two cells in which the chicken lamin B_2 protein is distributed throughout the cytoplasm.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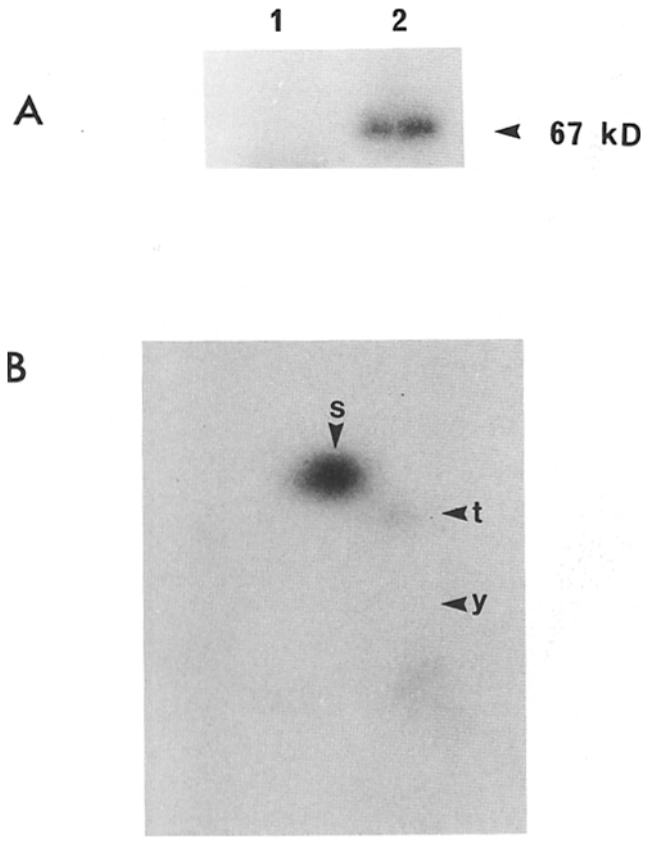

Figure 5. In vivo phosphorylation of chicken lamin B_2 expressed in fission yeast. (*A*) Chicken lamin B_2 was immunoprecipitated from ^{32}P-labeled yeast cells and analyzed using SDS-PAGE and autoradiography. Lane *1*, wild-type cells. Lane *2*, *LamB2* cells. A phosphoprotein of the correct molecular weight is detected only in *LamB2* (*arrow*). (*B*) Phosphorylated amino acids present in chicken lamin B_2 protein expressed in fission yeast. ^{32}P-labeled chicken lamin B_2 was immunoprecipitated and fractionated by SDS-PAGE as described above. The chicken lamin B_2 band was excised from the gel and subjected to acid hydrolysis. The resulting phosphoamino acids were separated by two dimensional electrophoresis (3). The positions of unlabeled standards as detected by ninhydrin staining are indicated (*s*, serine; *t*, threonine; *y*, tyrosine).

The results are shown in Fig. 4. Fig. 4 *A* and *C* show indirect immunofluorescence using anti-chicken lamin B_2 antibodies; Fig. 4 *B* and *D* show the same cells stained with the DNA-binding dye DAPI. As can be seen in the majority of the cells the ringlike structure associated with the nucleus characteristic of interphase cells (Fig. 2) has disappeared and is replaced by much more diffuse, punctate staining. In many of the cells (the cell indicated as *a* in Fig. 2 *A* is an average example), some staining is still roughly associated with the nucleus although in about 20% of the cells (for example, cell *b* in Fig. 2 *A*) no clear nuclear structure can be observed. Fig. 2 *C* and *D* show two cells of the latter type; here it can be seen that the anti-chicken lamin B_2 staining is punctate and dispersed throughout the cytoplasm.

In higher eukaryotes, disassembly of the nuclear lamina is also accompanied by an increase in extractability of the lamin protein. To see if an analogous change in extractability occurs in fission yeast we extracted *LamB2* cells arrested in mitosis as described above. However, in contrast to the situation in higher eukaryotes, the bulk of the chicken lamin B_2 protein remained insoluble even when the nuclear structure was dispersed as judged by immunofluorescence (data not shown). It is possible that we failed to observe solubilization of the lamins because complete disassembly of the nuclear structure only occurred in some of the cells. Alternatively, the cytological changes we observe may be due to a partial depolymerization of large lamina polymers, and filamentous fragments may remain entangled with cellular components. Indeed the cytoplasmic chicken lamin B_2 we observed in mitotically arrested fission yeast is found in discrete blobs rather than being finely dispersed as might be expected if the lamina had been completely solubilized (Fig. 4 *C*). Incomplete solubilization may be due to substoichiometric phosphorylation of the chicken lamin B_2 protein on some sites necessary for mitotic disassembly (see below).

A Cell Cycle–Regulated Lamin Kinase in Fission Yeast

In higher eukaryotes nuclear lamins are phosphorylated. Furthermore, at mitosis, the overall level of nuclear lamin phosphorylation increases, and phosphorylation on mitosis-specific residues is detected (6, 38, 40, 50). Phosphorylation on two of these sites has been shown to be required for mitotic disassembly of the nuclear lamina (13). To determine if dispersal of the structure we observed in fission yeast strains expressing chicken lamin B_2 could be due to similar events, the phosphorylation state of the chicken protein was

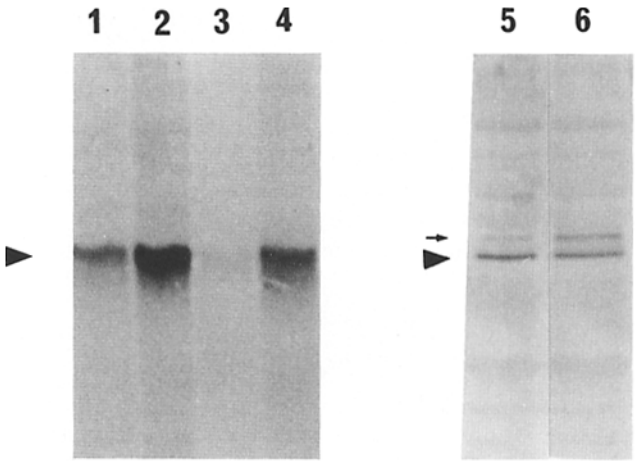

Figure 6. Increase in chicken lamin B_2 phosphorylation during fission yeast mitosis. (Lanes *1–4*) ^{32}P-labeled chicken lamin B_2 was immunoprecipitated from the indicated in vivo labeled samples and analyzed by SDS-PAGE and autoradiography. (Lane *1*) Asynchronous *LamB2* cells incubated at 35.5°C for 4 h; (lane *2*) *cdc13^{ts}LamB2* cells arrested in mitosis by incubation at 35.5°C for 4 h; (lane *3*) asynchronous *LamB2* cells incubated at 20°C for 5 h; (lane *4*) *nda3^{ts}LamB2* cells arrested in mitosis by incubation at 20°C for 5 h. Phosphorylated chicken lamin B_2 is indicated with an arrowhead. (Lanes *5* and *6*) Western blotting experiments of whole cell lysates made from *LamB2* (lane *5*) or *nda3^{ts}LamB2* (lane *6*) incubated at 20°C for 5 h. The major species of chicken lamin B_2 is indicated by an arrowhead; the small arrow indicates a minor species with reduced electrophoretic mobility. See Materials and Methods for full description of yeast strains.

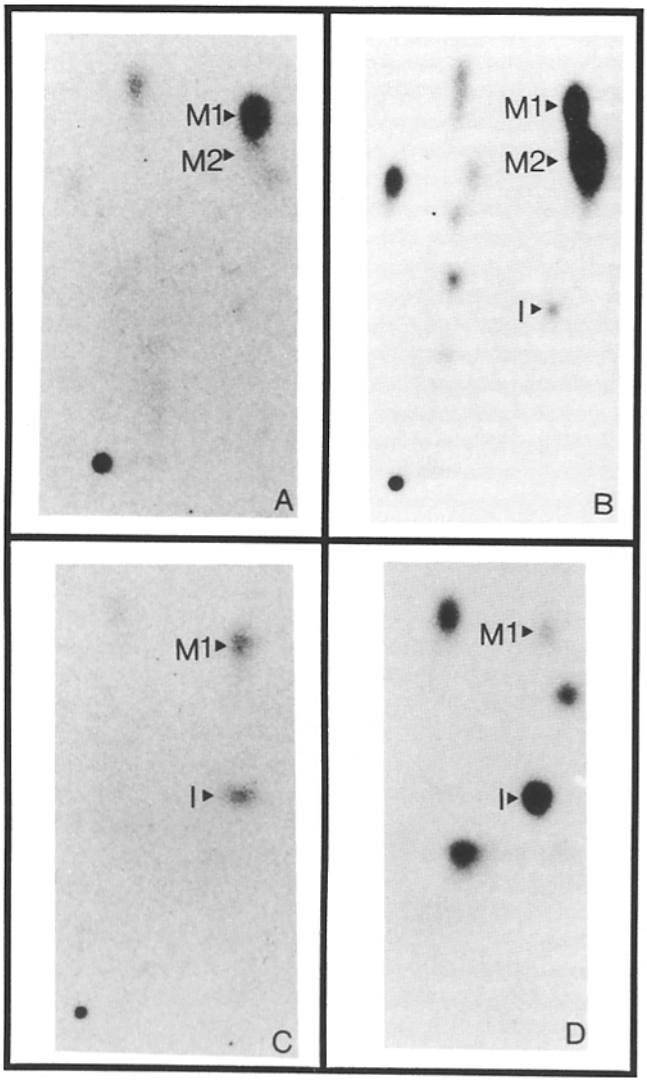

Figure 7. Tryptic phosphopeptide analysis of chicken lamin B_2 from interphase and mitotic chicken cells and interphase and mitotic fission yeast. ^{32}P phosphorylated chicken lamin B_2 was immunoprecipitated from the indicated in vivo labeled samples, eluted from gels, and digested with trypsin. The resulting phosphopeptides were separated by electrophoresis and chromatography as described in reference 40. (*A*) *cdc13ts LamB2* cells arrested in mitosis after incubation at 35.5°C for 4 h; (*B*) chick DU-249 cells arrested in mitosis as described in reference 40; (*C*) asynchronous *LamB2* cells cultured at 35.5°C as in (*A*); (*D*) interphase chick DU-249 cells. Mitosis-specific peptides detected in both yeast and chick cells are designated M1 and M2. An interphase phosphopeptide detected in both yeast and chick cells is designated *I*.

investigated. *LamB2* and wild-type cells were grown in the presence of inorganic ^{32}P-phosphate under the same conditions. Cells were lysed in RIPA buffer as described in Gould and Nurse (8) and the chicken lamin B_2 protein was immunoprecipitated using a monoclonal antibody directed against chicken lamin B_2 (20), and analyzed by SDS-PAGE and autoradiography. As shown in Fig. 5 *A*, this procedure detects a 67-kD phosphoprotein in *LamB2* cells (Fig. 5 *A*, lane *2*) but not in wild-type cells (Fig. 5 *A*, lane *1*). To determine which amino acids were phosphorylated, the ^{32}P-labeled chicken lamin B_2 protein was eluted from the gel and then hydrolyzed in 6 N HCl. The resulting phosphoamino acids were then separated by two-dimensional electrophoresis (3). As shown in Fig. 5 *B* the chicken lamin B_2 protein expressed in fission yeast was phosphorylated mainly on serine, although some phosphothreonine was also detected. No phosphotyrosine was observed. A similar pattern of amino acid phosphorylation has been reported for lamin B_2 in metaphase chicken cells (40).

We next investigated whether chicken lamin B_2 phosphorylation increases during mitosis. Mitotically arrested cells were generated using the strains *cdc13tsLamB2*, which arrests in mitosis at 36°C because of a defect in cyclin B (11), and *nda3csLamB2* (see above). Both strains were arrested in mitosis by incubation at the restrictive temperature in the presence of inorganic ^{32}P-phosphate. As controls, asynchronous cultures of *LamB2* cells were incubated under the same conditions. Cells were lysed and ^{32}P-labeled chicken lamin B_2 protein was analyzed as described above for Fig. 5. The results are shown in Fig. 6. Cells arrested in mitosis using either the *cdc13ts* mutation (Fig. 6, lane *2*) or the *nda3cs* mutation (Fig. 6, lane *4*) contain higher levels of phosphorylated chicken lamin B_2 than asynchronous control cultures incubated under the same conditions (Fig. 6, lane *1*; *LamB2* cells incubation as for *cdc13ts LamB2*; Fig. 6, lane *3*, *LamB2* cells incubation as for *nda3csLamB2* cells). Densitometric scanning of the autoradiogram reveals that the levels of phosphorylated chicken lamin B_2 increased 4.2-fold in the *cdc13tsLamB2* and 3.8-fold in *nda3csLamB2* relative to asynchronous cultures grown under the same conditions. Western-blotting experiments showed that the total amount of chicken lamin B_2 protein was the same in mitotic and asynchronous cultures. Fig. 6, lanes *5* and *6* show the results of a typical experiment. Roughly equal quantities of chicken lamin B_2 can be detected in *LamB2* (Fig. 6, lane *5*) and mitotically arrested *nda3csLamB2* (Fig. 6, lane *6*).

At present we have no information on the stoichiometry of phosphorylation of the chicken lamin B_2 protein at mitosis. However we note that a band of slightly lower electrophoretic mobility increases in intensity in the mitotically arrested cells (indicated by small arrow). The decrease in electrophoretic mobility could be because of phosphorylation of a particular mitotic site on a fraction (roughly 10–20%) of the chicken lamin B_2 protein. Substoichiometric phosphorylation of this site may explain why the interphasic structure is not completely dispersed, as judged by immunofluorescence, and why there is no increase in chicken lamin B_2 solubility in cells arrested in mitosis (see above). We note that in these strains, chicken lamin B_2 protein is expressed at comparatively high levels as it is under the control of the strong *adh* promoter. Such high levels of chicken lamin B_2 may result in formation of structures in which not all of the chicken protein is accessible to the mitotic kinase.

To compare the lamin kinase in fission yeast to the lamin kinase of higher eukaryotes, we analyzed the sites of lamin phosphorylation in fission yeast and compared them to the sites of phosphorylation in cultured chicken DU249 cells. ^{32}P-labeled chicken lamin B_2 protein was prepared from mitotically arrested and asynchronous cultures as described above. The phosphorylated chicken lamin B_2 protein was eluted from an SDS gel, digested with trypsin and the resulting peptides were separated in two dimensions by

electrophoresis and thin-layer chromatography. ^{32}P-labeled lamin B_2 protein prepared from either mitotically arrested or asynchronous cultures of chick DU249 cells were analyzed in parallel. The results of this analysis are shown in Fig. 7. In mitotically arrested fission yeast cells (Fig. 7 *A*) a peptide which is designated M1 is phosphorylated. A second peptide designated M2 is also phosphorylated to a lesser extent. These same two spots are also detected in ^{32}P-labeled chicken lamin B_2 from mitotically arrested chicken cells (Fig. 7 *B*). Mixing experiments were done to confirm that the fission yeast and chicken phosphopeptides comigrate (data not shown). The serine phosphorylated in the M1 peptide has been identified as Ser-16 (40) and mutation of the equivalent serine in the human lamin A protein is able to block mitotic disassembly of the nuclear lamina (13). In asynchronous fission yeast cells phosphorylation of the M1 and M2 peptides decreases substantially and a third phosphopeptide, designated I, is detected (Fig. 7 *C*). This resembles the changes in lamin phosphorylation observed in asynchronously growing cultured chicken cells (Fig. 7 *D*). Here also the levels of M1 and M2 peptide phosphorylation decrease, while phosphorylation of peptide I increases. Other peptides labeled to a lesser extent were also seen to be in common between yeast and chicken (compare Fig. 7, *A* and *B*, Fig. 7, *C* and *D*). These experiments show that the mitotic increase in phosphorylation of the chicken lamin B_2 protein expressed in fission yeast is due to qualitative and quantitative changes in lamin kinase activity. Moreover, in both mitotic and interphase fission yeast cells, the sites of phosphorylation are a subset of those phosphorylated in chicken cells suggesting that the fission yeast kinases are related to the chick kinases.

Phosphorylation of Chicken Lamin B_2 by p34^{cdc2} Kinase In Vitro

The above results demonstrate that fission yeast have a lamin kinase, indicating that this activity is evolutionarily conserved. Recent studies have shown that the cell cycle control protein p34^{cdc2}, a protein serine-threonine kinase that is activated at mitosis, is found in all eukaryotic cells (for review see reference 36) and biochemical studies have shown that p34^{cdc2} purified from starfish can phosphorylate nuclear lamins and induce lamina disassembly when incubated with isolated chick nuclei (40). It therefore seemed possible that the mitotic lamin kinase activity we detect in fission yeast could be p34^{cdc2}. To investigate this possibility, we took advantage of an in vitro assay for fission yeast p34^{cdc2} protein kinase (29). In this assay p34^{cdc2} protein kinase can be detected in crude yeast extracts by virtue of its ability to phosphorylate exogenous H1 histone. The activity can be shown to be because of p34^{cdc2} and not to other kinases because it is temperature sensitive in extracts prepared from *cdc2^{ts}* strains. Moreover, the temperature sensitivity of the activity in mutant extracts can be rescued by the addition of purified p13^{suc1} (29). p13^{suc1} is the product of the *suc1* gene and genetic and biochemical evidence suggests that it interacts physically with p34^{cdc2} (2, 12).

To investigate the role of p34^{cdc2} in chicken lamin B_2 phosphorylation we used the above assay system except that bacterially produced chicken lamin B_2 was added to the extracts instead of histone H1. Extracts were prepared from mitotically arrested fission yeast cells which either had a wild-type *cdc2* gene or a mutation known to result in temperature sensitivity of the *cdc2* kinase (see Materials and Methods). Substrate was prepared from extracts of *Escherichia coli* strains expressing the chicken lamin B_2 proteins. *E. coli* extracts were incubated first with the monoclonal antibody against chicken lamin B_2 and then with protein A conjugated to Sepharose beads (see Materials and Methods). The beads were then washed and incubated with extracts prepared from various yeast strains in the presence of γ^{32}P-ATP for 15–30 min at either 25 or 36°C. At the end of the incubation period, the beads were washed, boiled in SDS-PAGE sample buffer, and then the ^{32}P-labeled proteins were analyzed by gel electrophoresis and autoradiography (Fig. 8 *A*). Histone H1 kinase assays of the same extracts were carried out in parallel (Fig. 8 *B*).

When extracts from yeast carrying a wild-type *cdc2* gene were used, phosphorylation of a 67-kd protein was detected (Fig. 8 *A*, lanes *1* and *2*). This phosphoprotein was absent when the assay was done using extracts from bacteria that didn't express chicken lamin B_2 (data not shown). The level of kinase activity was roughly the same whether the assay was carried out at 25° (Fig. 8 *A*, lane *1*) or 36°C (Fig. 8 *A*, lane *2*). However in strains bearing a temperature-sensitive mutation in the *cdc2* gene, lamin kinase activity at 36°C (Fig. 8 *A*, lane *4*) was significantly lower than lamin kinase activity at 25°C (Fig. 8 *B*, lane *3*). However if this extract was supplemented with bacterially produced p13^{suc1}, kinase activity at 36°C was rescued (Fig. 8 *A*, lane *5*). As has previously been shown (29), histone H1 kinase activity in these extracts showed a similar pattern of temperature sensitivity and rescue by p13^{suc1} (Fig. 8 *B*, lanes *3–5*). Tryptic peptide mapping of the in vitro phosphorylated chicken lamin B_2 protein revealed that both the M1 and M2 sites were phosphorylated (data not shown).

Based on these results we conclude that the mitotic lamin kinase we detect in fission yeast is either p34^{cdc2} itself or a kinase that is very directly dependent on p34^{cdc2} for activity. To investigate this possibility further we immunoprecipitated p34^{cdc2} from mutant and wild-type extracts and then assayed the immunoprecipitates for lamin-kinase activity. As shown in Fig. 8 *C*, high levels of lamin kinase activity could be detected in immunoprecipitates from wild-type cells (Fig. 8 *C*, lane *4*). This activity was reduced over sevenfold in immunoprecipitates of extracts containing a temperature sensitive cdc2 protein (Fig. 8 *C*, lane *3*). Both immunoprecipitates contained equal amounts of p34^{cdc2} (data not shown). Lamin-specific kinase activity was not detected in either extract when immunoprecipitates were made using preimmune sera (Fig. 8 *C*, lanes *1* and *2*). From these experiments we conclude that p34^{cdc2} is likely to be directly responsible for mitotic phosphorylation of the chicken lamin B_2 protein.

Discussion

The Role of p34^{cdc2} in Mitotic Events

The protein kinase p34^{cdc2} controls entry into mitosis in all eukaryotic cells. Entry into mitosis correlates with the appearance of many new phosphoproteins (16, 21, 23). By analogy with control of other cellular processes by protein phosphorylation, p34^{cdc2} could initiate mitosis by activating a

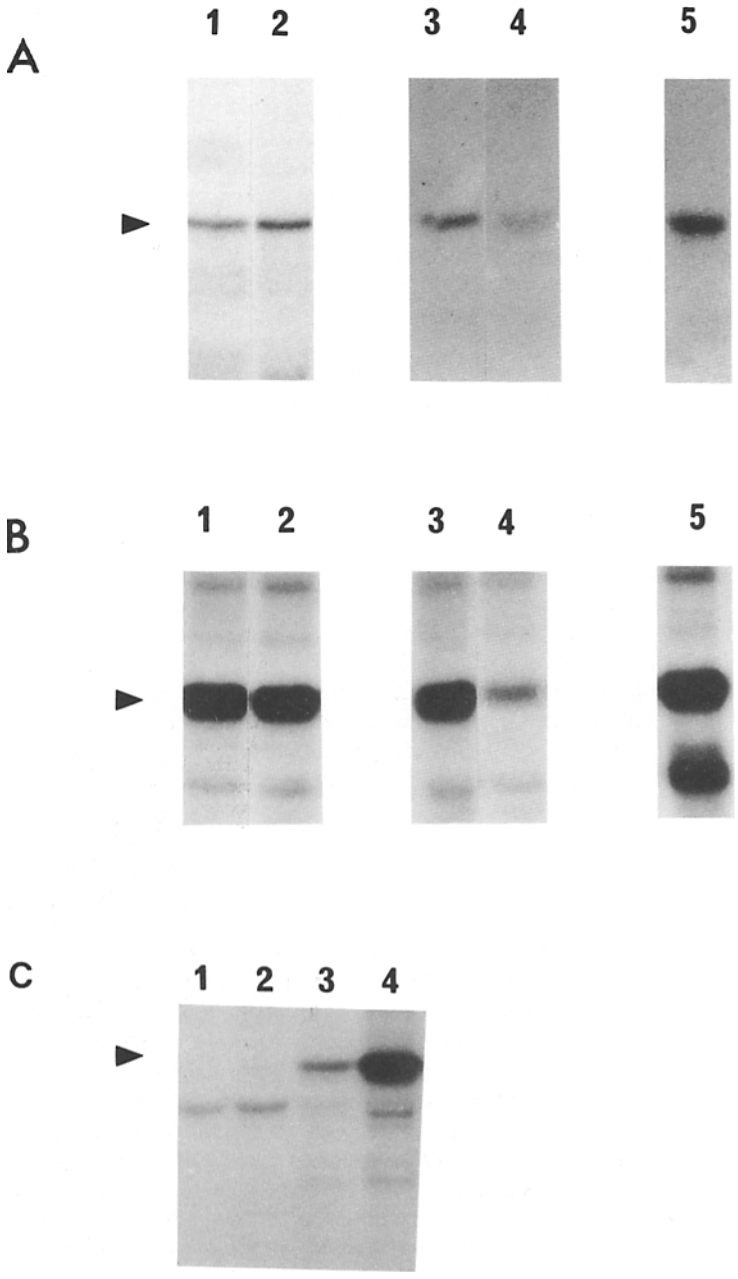

Figure 8. p34^{cdc2} is directly responsible for phosphorylation of chicken lamin B_2 in vitro. (*A* and *B*) Mitotic extracts from strains with wild-type or temperature sensitive *cdc2* proteins were assayed for lamin kinase activity (*A*) or H1 histone kinase activity (*B*). For lamin kinase assays bacterially produced chicken lamin B_2 was immunoprecipitated using protein A-Sepharose beads, and the beads were incubated with yeast extracts at the indicated temperatures in the presence or absence of bacterially produced p13^{suc1}. At the end of the incubation the beads were washed, boiled in SDS-PAGE sample buffer and phosphorylation of chicken lamin B_2 was analyzed by SDS-PAGE and autoradiography. H1 histone kinase assays were performed as described in reference 29. The arrow in panel *A* indicates the position of phosphorylated chicken lamin B_2. The arrowhead in *B* indicates the position of phosphorylated Histone H1. Lane *1*, wild-type extract assayed at 25°C; lane *2*, wild-type extract assayed at 35.5°C; lane *3*, *cdc2^{ts}* extract assayed at 25°C; lane *4*, *cdc2^{ts}* extract assayed at 35.5°C; lane *5*, *cdc2^{ts}* extract assayed at 36°C in the presence of 0.5 μM p13^{suc1}. (*C*) Lamin kinase assays of immunoprecipitates using preimmune and anti-p34^{cdc2} antiserum. Lane *1*, preimmune control, *cdc2^{ts}* extract; lane *2*, preimmune control, wild-type extract; lane *3*, p34^{cdc2} immunoprecipitate from a *cdc2^{ts}* extract; lane *4*, p34^{cdc2} immunoprecipitate from a wild-type extract. Phosphorylated chicken lamin B_2 is indicated by an arrowhead. All assays were performed at 36°C.

cascade of secondary kinases which would in turn phosphorylate mitotic substrates. Alternatively, p34^{cdc2} could initiate mitosis by directly phosphorylating key mitotic substrates.

We have investigated the role of p34^{cdc2} in mitotic protein phosphorylation by expressing a well-characterized mitotic substrate, chicken nuclear lamin B_2, in fission yeast. We have used the resulting strains to show that fission yeast contain a mitosis-specific lamin kinase that phosphorylates chicken lamin B_2 proteins on the same sites as a lamin kinase detected in mitotic chick cells. Moreover, phosphorylation by the fission yeast kinase correlates with a striking change in the intracellular distribution of the chicken lamin B_2 at mitosis. We conclude that fission yeast contain a mitosis-specific lamin kinase that resembles the lamin kinase of higher eukaryotes.

The fission yeast lamin kinase can be detected in vitro in crude extracts prepared from mitotically arrested cells. We find that the kinase activity is temperature sensitive in extracts prepared from yeast strains having mutations that render the p34^{cdc2} protein kinase inactive at 36°C in vitro (29). Moreover, the temperature sensitivity is rescued by addition of p13^{suc1}, the protein product of *sucl*, a gene that suppresses the temperature sensitivity of *cdc2^{ts}* mutants in vivo (12) and of the kinase activity in vitro (29) in an allele-specific manner. These results indicate that p34^{cdc2} is likely to be the fission yeast lamin kinase. It is formally possible that we are detecting a distinct kinase which is being dynamically maintained in an active state by p34^{cdc2} in the extracts. However, this seems unlikely for a number of reasons. Firstly, the extract is prepared from mitotically arrested cells. Thus, a downstream kinase component of a p34^{cdc2}-dependent cascade should already be active, and indeed lamin kinase activity can be detected when the extracts are

assayed at 25°C. The extract is prepared using a buffer that contains phosphatase inhibitors which should prevent inactivation of the kinase by dephosphorylation. Secondly, as the extract is substantially more dilute than the cytoplasm of intact cells, the protein concentrations are probably not high enough to reconstitute a $p34^{cdc2}$-dependent kinase cascade. Finally, a lamin kinase activity that is temperature sensitive in cdc2 mutants can be specifically immunoprecipitated using anti-$p34^{cdc2}$ antibodies.

As the fission yeast kinase resembles the kinase of higher eukaryotes, we propose that $p34^{cdc2}$ is also responsible for nuclear lamin phosphorylation in higher eukaryotes. This confirms the recent finding that highly purified $p34^{cdc2}$ prepared from starfish can directly phosphorylate chicken nuclear lamins and is sufficient to induce nuclear lamina disassembly when incubated with chick nuclei in vitro (40). The genetic approach we have used complements and extends this earlier study because we have shown that mutations that alter the *cdc2* protein directly affect lamin kinase activity. This excludes the possibility that the previously obtained results were due to an unrelated kinase in the chick nuclei or a contaminant in the starfish $p34^{cdc2}$ kinase preparation. Conversely, our present results do not rigorously exclude the possibility that a distinct kinase that is part of a $p34^{cdc2}$ complex is responsible for lamin phosphorylation. However, the previous studies with purified starfish $p34^{cdc2}$ suggest that this explanation is unlikely as lamin kinase activity was detected in a purified complex shown to consist of only two major components, $p34^{cdc2}$ and cyclin B (19).

Purified starfish $p34^{cdc2}$ also appears to directly phosphorylate the nucleolar proteins nucleolin and NO38 (1b,39). Thus, it is plausible that $p34^{cdc2}$ induces at least some mitotic events by directly phosphorylating key structural proteins rather than by triggering a cascade of secondary kinase reactions (for review and discussion see references 28 and 40).

A Nuclear Lamina-like Structure in Fission Yeast?

Immunofluorescence of fission yeast cells expressing chicken lamin B_2 revealed that the protein is found in a ringlike structure associated with the fission yeast nucleus. Fractionation studies show that this structure is resistant to extraction with detergent and high salts. Thus chicken lamin B_2 protein expressed in yeast appears to be in a structure with some similarities to the nuclear lamina of higher eukaryotes. We suggest that the chicken protein may be interacting with endogenous yeast proteins and becoming incorporated into a yeast structure analogous to the nuclear lamina of higher eukaryotes. In support of this possibility, lamin modifying activities similar to those found in higher eukaryotes were also detected in fission yeast. These include a kinase that phosphorylates chicken lamin B_2 during interphase (Fig. 7) and an activity, most likely a farnesyltransferase, that increases the electrophoretic mobility of chicken lamin B_2, (Fig. 1), as well as the mitotic lamin kinase $p34^{cdc2}$. As yet neither nuclear lamins or intermediate filament proteins have been identified in fission yeast. However, budding yeast have proteins immunologically related to nuclear lamins, and these proteins can interact with vertebrate nuclear lamina components in vitro (5). If similar interactions can occur in vivo, the structure we observe in *LamB2* may be the fission yeast nuclear lamina.

Alternatively, the chicken lamin B_2 protein by itself may be sufficient for assembly of a nuclear lamina. However, we do not detect any novel structures in the *LamB2* strain by light microscopy, and the strain is able to grow and divide at normal rates. One might expect a nonphysiological insoluble structure to have a more noticeable effect on cell morphology or viability. We also note that we have no direct evidence that the chicken lamin B_2 protein is intranuclear; the immunofluorescence data are also consistent with it being found in a cytoplasmic structure that forms around the nucleus during fixation. Excluding this possibility will require detailed studies of the *LamB2* strain using ultrastructural techniques. Clear proof that yeast contain lamins ultimately requires identification of fission yeast genes that could encode intermediate filament or nuclear lamin proteins.

The existence of a nuclear lamina-like structure in fission yeast would be compatible with a number of studies showing that lower eukaryotes contain structures resembling the karyoskeleton of high eukaryotes. As already noted, the budding yeast, *S. cerevisiae*, contains proteins immunologically related to nuclear lamins (5), and to nuclear pore components (4, 33). In addition, a structure that resembles the higher eukaryotic nuclear matrix and may interact with chromatin in an analogous manner has been identified (1,a). Also, the budding yeast protein REP2, which plays a role in segregation of the 2-μm circle, has some similarity to intermediate filament proteins and is found associated with an insoluble nuclear fraction (51).

The nuclear-associated structure we detect in the *LamB2* strain is reorganized during mitosis when chicken lamin B_2 protein is phosphorylated by $p34^{cdc2}$, becoming dispersed throughout the cytoplasm. This is surprising as fission yeast mitosis takes place in the absence of nuclear membrane breakdown. Dispersal could be explained if the chicken protein is extranuclear (see above) and is held is place by components of the cytoskeleton that reorganize at mitosis. Investigating this possibility will require ultrastructural studies of the mitotic and interphase structures that we have observed. However, it is worth considering the possibility that a correctly located lamina-like structure may be able to disassemble in the absence of nuclear envelope breakdown. Pachytene meiotic cells have been shown to lack a nuclear lamina although the nuclear membrane is still intact (43) and lamina disassembly in the absence of nuclear envelope breakdown has also been observed in vitro in nuclei added to cell-free extracts that support mitosis (34, 40). Possibly the nuclear membrane can be disrupted sufficiently to allow lamina disassembly without completely breaking down. Partially disassembled "fenestrated" nuclear membranes are a feature of mitosis in some fungi (52) and have also been observed in mitotic cells of the thymus (30). Perhaps the mitotic nuclear membrane of fission yeast contains "gaps" which are too small or infrequent to have been detected in previous ultrastructural studies (24, 45) but which still permit disassembly of the yeast karyoskeleton. Disassembly of a karyoskeleton may be necessary to accommodate the rapid distortion of nuclear shape that takes place as the nucleus extends along the length of the cell and then divides in two (24, 45, 46). The contrasting modes of mitosis in yeast and animal cells could be points along a continuum of degrees of nuclear envelope breakdown, rather than mechanistically distinct pro-

cesses. It may be possible to use fission yeast strains expressing vertebrate lamins to explore this possibility further.

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Note Added in Proof: Similarity between nuclear lamins and another *S. cerevisiae* protein, the product of the SIR4 gene, has recently been reported (Diffley, J. F. X., and B. Stillman. 1990. *Nature [Lond.].* 342:24).

References

1*a*. Amati, B., and S. Gasser. 1988. Chromosomal ARS and CEN elements bind specifically to the yeast nuclear scaffold. *Cell.* 54:967–978.

1*b*. Belenguer, P., Caizergues-Ferrer, M., Labbé, J.-C., Dorée, M., Amalrie, F. 1990. Mitosis-specific phosphorylation of nucleolin by p34cdc2 protein kinase. *Mol. Cell. Biol.* 10:3607–3618.

2. Brizuela, L., G. Draetta, and D. Beach. 1987. $p13^{suc1}$ acts in the fission yeast cell division cycle as a component of the $p34^{cdc2}$ protein kinase. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3507–3514.

3. Cooper, J. A., B. M. Sefton, and T. Hunter. 1983. Detection and quantification of phosphotyrosine in proteins. *Methods Enzymol.* 99:387–402.

4. Davis, L. I., and G. R. Fink. 1990. The NUP1 gene encodes an essential component of the yeast nuclear pore complex. *Cell.* 61:965–978.

5. Georgatos, S., I. Maroulakou, and G. Blobel. 1989. Lamin A, lamin B, and lamin B receptor analogues in yeast. *J. Cell Biol.* 108:2069–2072.

6. Gerace, L., and G. Blobel. 1980. The nuclear envelope lamina is reversibly depolymerized during mitosis. *Cell.* 19:277–287.

7. Gerace, L., and B. Burke. 1988. Functional organization of the nuclear envelope. *Annu. Rev. Cell Biol.* 4:335–374.

8. Gould, K. L., and P. Nurse. 1989. Tyrosine phosphorylation of the fission yeast $cdc2^+$ protein kinase regulates entry into mitosis. *Nature (Lond.).* 342:39–45.

9. Gould, K. L., S. Moreno, N. K. Tonks, and P. Nurse. 1990. A human protein-tyrosine phosphatase can replace the fission yeast mitotic activator, $p80^{cdc25}$ *in vivo* and activates $p34^{cdc2}$ in vitro and in vivo. *Science (Wash. DC).* 250:1573–1576.

10. Hagan, I. M., and J. S. Hyams. 1988. The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast *Schizosaccharomyces pombe. J. Cell Sci.* 89:343–357.

11. Hagan, I. M., J. Hayles, and P. Nurse. 1988. Cloning and sequencing of the cyclin-related $cdc13^+$ gene and a cytological study of its role in fission yeast mitosis. *J. Cell Sci.* 91:587–595.

12. Hayles, J., D. Beach, B. Durkacz, and P. Nurse. 1986*a*. The fission yeast cell cycle control gene $cdc2^+$: isolation of a sequence $suc1^+$ that suppresses *cdc2* mutant function. *Mol. Gen. Genet.* 202:291–293.

13. Heald, R., and F. McKeon. 1990. Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell.* 6:579–589.

14. Hiraoka, Y., T. Toda, and M. Yanagida. 1984. The $nda3^+$ gene of fission yeast encodes β-tubulin: a cold sensitive nda3 mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell.* 39:349–358.

15. Holz, D., R. A. Tanaka, J. Hartwig, and F. McKeon. 1989. The CaaX motif of lamin A functions in conjunction with the nuclear localization signal to target assembly to the nuclear envelope. *Cell.* 59:969–977.

16. Karsenti, E., R. Bravo, and M. W. Kirschner. 1987. Phosphorylation changes associated with early cycle in Xenopus eggs. *Dev. Biol.* 119:423–453.

16*a*. Kitten, G., E. Nigg. 1991. The CaaX motif is required for isoprenylation, carboxyl methylation and nuclear membrane association of lamin B2. *J. Cell Biol.* In press.

17. Kohli, J. 1987. Genetic nomenclature and gene list of the fission yeast *Schizosaccharomyces pombe. Curr. Genet.* 11:575–589.

18. Krohne, G., I. Waizenegger, and T. H. Hoger. 1989. The conserved carboxy-terminal cysteine of nuclear lamins is essential for lamin association with the nuclear envelope. *J. Cell Biol.* 109:2003–2011.

19. Labbé, J. C., J. P. Capony, D. Caput, J. C. Cavadore, J. Derancourt, M. Kaghad, J.-M. Lelias, A. Picard, and M. Dorée. 1989. MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing 1 molecule of cdc2 and 1 molecule of cyclin B. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3053–3058.

20. Lehner, C. F., V. Kurer, H. Eppenberger, and E. Nigg. 1986. The nuclear lamin protein family in higher vertebrates: identification of quantitatively minor lamin proteins by monoclonal antibodies. *J. Biol. Chem.* 261:13293–13301.

21. Lohka, M. J., J. L. Kyes, and J. L. Maller. 1987. Metaphase protein phosphorylation in *Xenopus laevis* eggs. *Mol. Cell. Biol.* 7:760–768.

22. MacNeill, S. A., and P. Nurse. 1989. Genetic interactions in the control of mitosis in fission yeast. *Curr. Genet.* 16:1–6.

23. Maller, J. L., and D. S. Smith. 1985. Two-dimensional polyacrylamide gel analysis of changes in protein phosphorylation during maturation of *Xenopus* oocytes. *Dev. Biol.* 109:150–156.

24. McCully, E. K., and C. F. Robinow. 1971. Mitosis in the fission yeast *S. pombe*: a comparative study with light and electron microscopy. *J. Cell Sci.* 9:475–507.

25. McIntosh, J. R., and M. P. Koonce. 1989. Mitosis. *Science (Wash. DC).* 246:622–628.

26. Miake-Lye, R., and M. Kirschner. 1985. Induction of early mitotic events in a cell-free system. *Cell.* 41:165–175.

27. Mitchison, J. M. 1970. Physiological and cytological methods for *Schizosaccharomyces pombe. Methods Cell Physiol.* 4:131–146.

28. Moreno, S., and P. Nurse. 1990. Substrates for $p34^{cdc2}$: in vivo veritas? *Cell.* 61:549–551.

29. Moreno, S., J. Hayles, and P. Nurse. 1989. Regulation of $p34^{cdc2}$ protein kinase during mitosis. *Cell.* 58:361–372.

30. Murray, R. G., A. S. Murray, and A. Pizzo. 1965. The fine structure of mitosis in rat thymic lymphocytes. *J. Cell Biol.* 26:601–619.

31. Nakagawa, J., G. T. Kitten, and E. A. Nigg. 1989. A somatic cell-derived system for studying both early and late mitotic events in vitro. *J. Cell Sci.* 94:449–462.

32. Nasmyth, K., and P. Nurse. 1981. Cell division cycle mutants altered in DNA replication and mitosis in the fission yeast *Schizosaccharomyces pombe. Mol. Gen. Genet.* 182:119–124.

33. Nehrbass, U., H. Kern, A. Mutvei, H. Horstmann, B. Marshallsay, and E. C. Hurt. 1990. NSP1: a yeast nuclear envelope protein localized at the nuclear pores exerts it essential function by its carboxy-terminal domain. *Cell.* 61:979–989.

34. Newport, J. W., and T. Spann. 1987. Disassembly of the nucleus in mitotic extracts: membrane vesicularization, lamin disassembly and chromosome condensation are independent processes. *Cell.* 48:219–230.

35. Nigg, E. A. 1989. The nuclear envelope. *Curr. Opinions Cell Biol.* 1:435–440.

36. Nurse, P. 1990. Universal control mechanism regulating onset of M-phase. *Nature (Lond.).* 344:503–508.

37. Nurse, P., P. Thuriaux, and K. A. Nasmyth. 1976. Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe. Mol. Gen. Genet.* 146:167–178.

38. Ottaviano, Y., and L. Gerace. 1985. Phosphorylation of the nuclear lamins during interphase and mitosis. *J. Biol. Chem.* 260:624–632.

39. Peter, M., J. Nakagawa, M. Doree, J. C. Labbe, and E. A. Nigg. 1990*a*. Identification of major nucleolar proteins as candidate mitotic substrates of cdc2 kinase. *Cell.* 60:791–801.

40. Peter, M., J. Nakagawa, M. Dorée, J. C. Labbe, and E. A. Nigg. 1990*b*. In vitro disassembly of the nuclear lamina and M phase-specified phosphorylation of lamins by cdc2 kinase. *Cell.* 61:591–602.

41. Rosenberg, A. H., B. N. Lade, D. Chui, S. W. Lin, J. J. Dunn, and F. W. Studier. 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. *Gene (Amst.).* 56:125–130.

42. Russell, P., and P. Nurse. 1986. *cdc25+* functions as a mitotic inducer in the mitotic control of fission yeast. *Cell.* 45:145–153.

43. Stick, R., and H. Schwarz. 1983. Disappearance and reformation of the nuclear lamina structure during specific stages of meiosis in oocytes. *Cell.* 33:949–958.

44. Stick, R., B. Angres, C. Lehner, and E. A. Nigg. 1988. The fates of chicken nuclear lamin proteins during mitosis: evidence for a reversible redistribution of lamin B2 between inner nuclear membrane and elements of the endoplasmic reticulum. *J. Cell Biol.* 107:397–406.

45. Tanaka, K., and T. Kanbe. 1986. Mitosis in the fission yeast *S. pombe* as revealed by freeze-substitution electron microscopy. *J. Cell Sci.* 80: 253–268.

46. Toda, T., M. Yamamoto, and M. Yanagida. 1981. Sequential alterations in the nuclear chromatin region during mitosis of the fission yeast *S. pombe*: video fluorescence microscopy of synchronously growing wild-type and cold-sensitive *cdc* mutants by using a DNA-binding probe. *J. Cell Sci.* 52:271–287.

47. Toda, T., K. Umesono, and A. Hiraoka. 1983. Cold sensitive nuclear division arrest mutants of the fission yeast *Schizosaccharomyces pombe. J. Mol. Biol.* 168:251–270.

48. Vorburger, K., C. F. Lehner, G. T. Kitten, H. M. Eppenberger, and E. A. Nigg. 1989*a*. A second higher vertebrate B-type lamin: cDNA sequence determination and in vitro processing of chicken lamin B2. *J. Mol. Biol.* 208:415–415.

49. Vorburger, K., G. T. Kitten, and E. A. Nigg. 1989*b*. Modification of nuclear lamin proteins by a mevalonic acid derivative occurs in reticulocyte lysates and requires the cysteine residue of the C-terminal CXXM motif. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:4007–4013.

50. Ward, G. E., and M. W. Kirschner. 1990. Identification of cell cycle-regulated phosphorylation sites on nuclear lamin C. *Cell.* 61:561–577.

51. Wu, L.-C. C., P. A. Fisher, and J. R. Broach. 1987. A yeast plasmid partitioning protein is a karyoskeletal component. *J. Biol. Chem.* 262:883–891.

52. Zickler, D. 1981. Mechanisms of nuclear division in fungi. *In* The Fungal Nucleus. K. Gull and S. G. Oliver, editors. Cambridge University Press, Cambridge, UK. 85–113.